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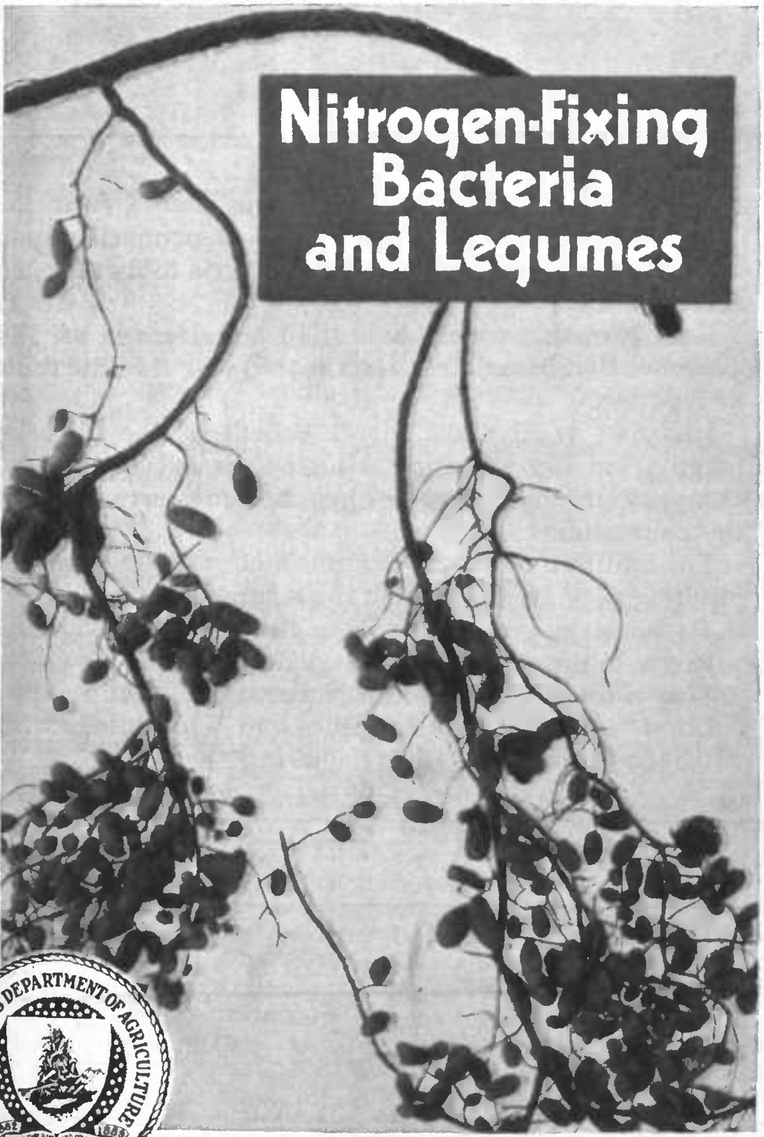
Nitrogen-Fixing Bacteria and Legumes



NITROGEN-FIXING BACTERIA AND LEGUMES

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INTRODUCTION

Crops such as alfalfa, red clover, soybean, vetch, bean, and others belonging to the pea family are commonly called legumes or leguminous plants. They have been grown by farmers since the dawn of agriculture, and at present many species are planted extensively all over the world. Under natural conditions, also, legumes occupy a prominent place. Their importance, aside from the many economic uses to which they are put, lies mainly in the fact that they are usually richer in proteins than other plants and that much of the nitrogen in these proteins comes from the atmosphere.

Legumes are essential to a well-rounded agricultural program, since by their high nitrogen content they balance the carbohydrates of cereal and root crops. Plants that are not legumes (or nonlegumes, as they are called) derive their supply of nitrogen entirely from the soil. Legumes may take nitrogen from the soil also, but in the presence of certain bacteria, which form characteristic growths on their roots, they have access to the nitrogen of the air. The plant houses and feeds the bacteria while they, through some unexplained process, carry on nitrogen fixation. Without bacteria legumes take no nitrogen from the air, and without legumes these bacteria are of no appreciable benefit to the soil; to perform satisfactorily the nitrogen-fixing function, the plants and bacteria must work together.

The root growths in which these bacteria live on legumes are called nodules or tubercles, hence the bacteria are commonly known as root-nodule bacteria, legume bacteria, and sometimes as rhizobia. Root-nodule bacteria are very minute organisms, visible as a mass in nodules and singly only by means of powerful microscopes.

Most farmers know that available nitrogen in the soil is necessary for the satisfactory growth of crops, that nitrogen is easily lost from the soil by leaching, and that nitrogen fertilizers are expensive to buy. In some few instances nature may have provided nitrogen in abun-

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dance, but in most soils, particularly those in humid sections, the supply is always limited and must be supplemented from time to time if fertility is to be maintained. This may be accomplished by additions of mineral nitrogen, many forms of which may be purchased, by additions of organic matter consisting of plant and animal residues, or by additions of atmospheric nitrogen made available through the activities of legumes and their bacteria. The nitrogen combined in legume tissues is in organic form and as such is not immediately available when plowed under. After legumes are plowed under, other groups of soil bacteria, universally distributed, assume the task of gradually decomposing the organic nitrogen into an available form, which may be used by either legumes or nonlegumes.

ROOT NODULES, THE SEAT OF NITROGEN FIXATION

If nodule organisms are abundant in the soil, legume roots spreading in that soil come in contact with them early in their growth. These bacteria, being specially adapted for growth on the legume roots, spread over them. If conditions are favorable nodules begin to appear in about 15 days after seed germination (fig. 1). While contact between bacteria and root is necessary for the starting of nodules, favorable soil conditions are also essential to their establishment.

Since nodules are the principal superficial indication that nitrogen-fixing legume bacteria are present, everyone interested in agricultural work should be familiar with them. True nitrogen-fixing nodules should not be confused with galls or false nodules initiated by disease bacteria and nematodes which, fortunately, occur less constantly than legume nodules. Disease growths of this type are shown in figure 2.

At the approach of maturity and sometimes during dry periods nodules disappear. With this in mind, examinations for nodules should preferably be made when plants are young and vigorous and after moisture has been reasonably plentiful in the soil for a short time. Since nodules are easily detached from roots, care must be exercised in taking them from the soil. In light, sandy soils it is usually sufficient to raise roots with surrounding soil and dislodge the latter by gentle shaking. A spade or other sharp digging tool is useful in raising soil and roots. Plants in heavy soils should be lifted with a spade, and the resulting soil block soaked or sprayed with water to expose the roots. To obtain a general idea of the uniformity of nodules on plants in a planting, samples should be examined in several parts of the field.

Nodules may be small or large, in accordance with age and the species upon which they are formed; those on red clover (fig. 3, *A*) are relatively small, on soybean and cowpea they are of intermediate size, while on velvetbean they may attain the size of a hulled black walnut (fig. 3, *B*). Shape also varies with species. Soybean and cowpea nodules are usually round and pealike (fig. 4, *A*). Those of vetch, alfalfa, red clover, peas, and velvetbean are rod- or club-shaped when young, later under some conditions forking at the unattached end, forming pear-shaped growths. Sometimes the forking continues, and large nodules with many lobes result. Examples of lobed nodules are shown in figures 3, *B*, and 4, *B*.

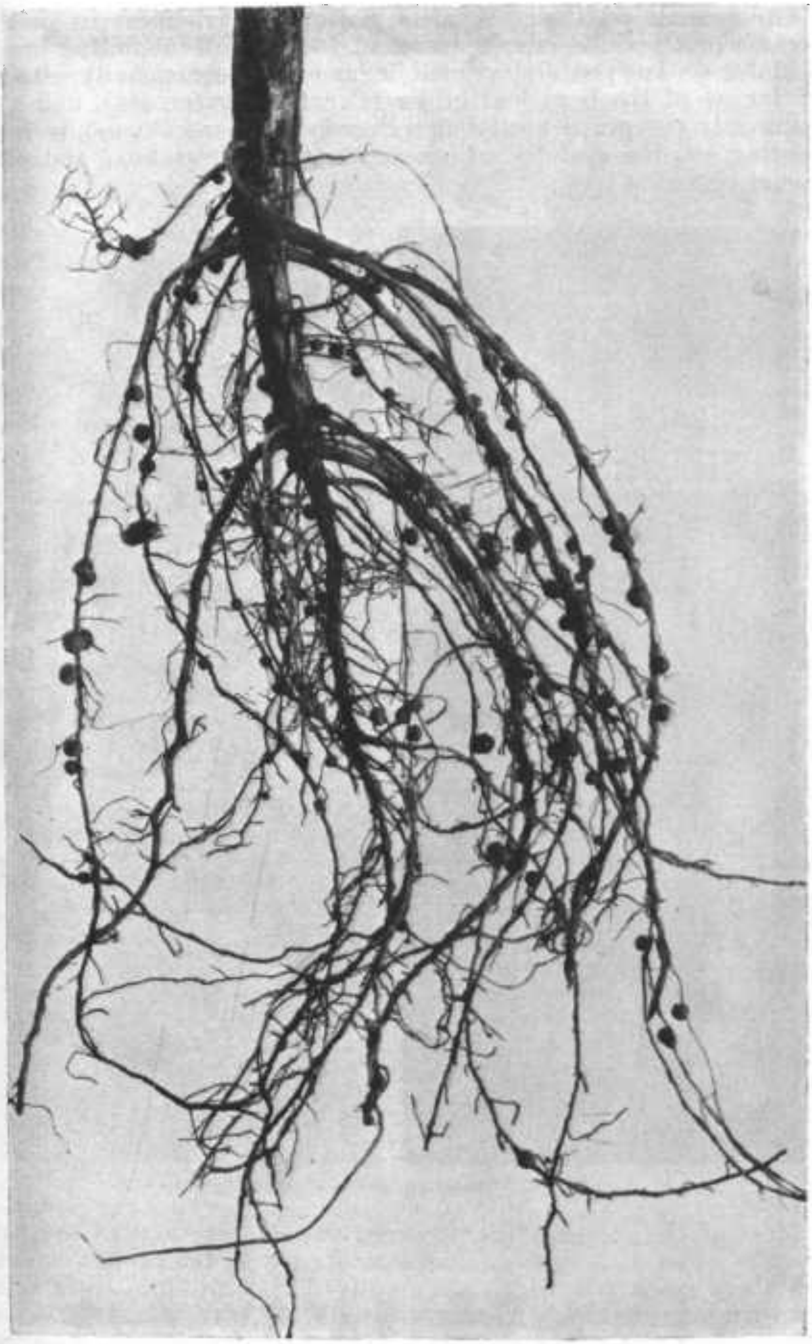

FIGURE 1.—Soybean root with nodules in usual abundance.

On account of their succulent nature and richness in protein, legume root nodules offer a source of food to soil-inhabiting insects. Nodules on cowpea and related legumes are occasionally attacked by larvae of the bean leaf beetle (*Ceratoma trifurcata*), and quite commonly the grape mealybug (*Pseudococcus maritimus*) is found feeding on the nodules of clover, soybean, lespedeza, and other legumes (fig. 5).

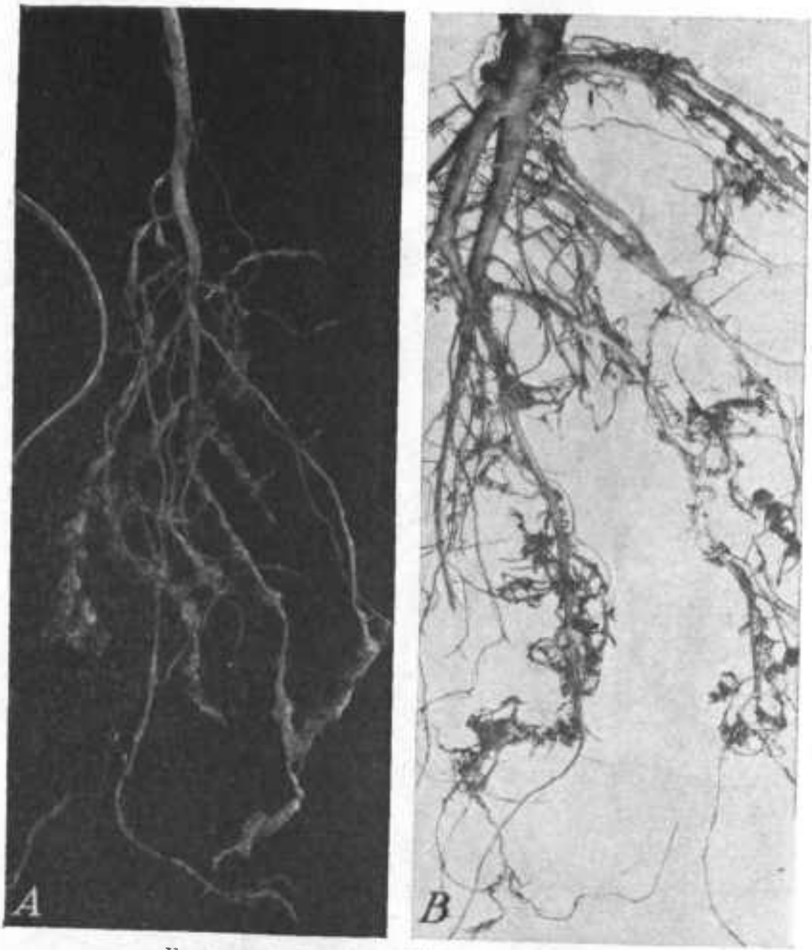

FIGURE 2.—*A*, Nematode galls; *B*, bacterial galls.

Most of the common wild or cultivated leguminous plants form nodules when their roots come into contact with the proper bacteria, but there are a few which apparently do not produce them under known circumstances. Among these are the honeylocust (*Gleditsia triacanthos*), Kentucky coffeetree (*Gymnocladus dioica*), American redbud (*Cercis canadensis*), and negro coffee (*Cassia occidentalis*). None of these are agriculturally important.

On some nonlegumes normal root growths, resembling superficially the nodules of legumes, are found. Two individual specimens of these are shown in figure 6. They regularly occur on such common

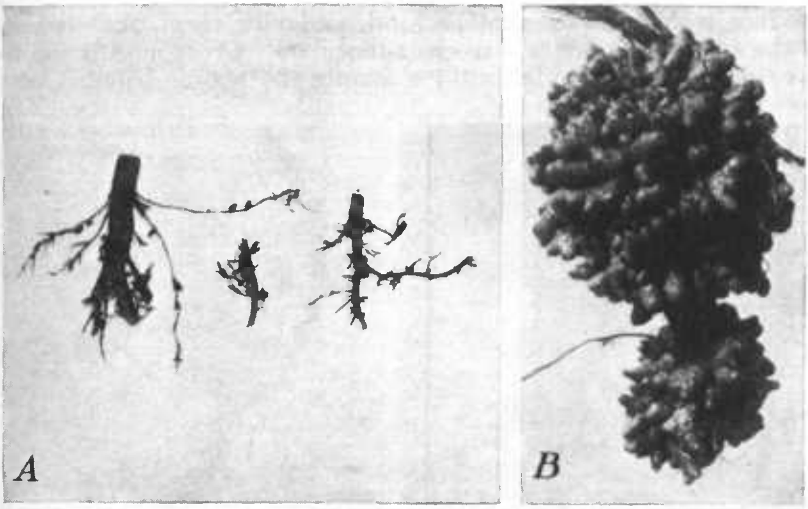

FIGURE 3.—A, Red clover nodules; B, velvetbean nodules.

wild nonlegumes as alders, Jersey-tea (*Ceanothus americanus*), russet buffaloberry (*Lepargyrea canadensis*), horsetail-tree (*Casuarina equisetifolia*), and the cycads. The function of these nodules is not well understood, but they apparently are necessary for the nutrition of their host.

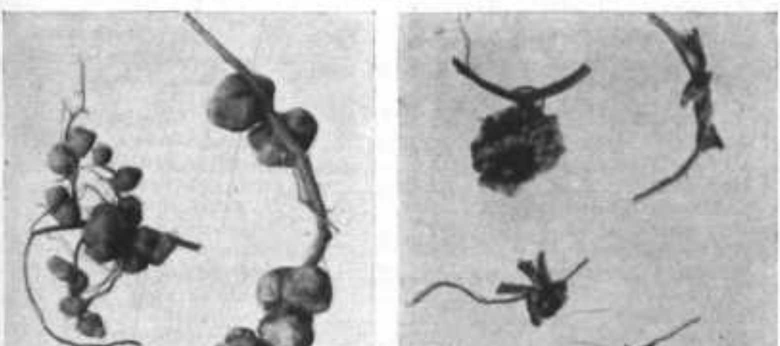

FIGURE 4.—A, Cowpea nodules; B, alfalfa nodules.

NODULE BACTERIA NOT ALL THE SAME

All nodule bacteria have the same general function, but they are not identical in their ability to produce nodules on various species

of legumes. This divides them into kinds or species. Some species cause nodules to form on the roots of a wide range of leguminous plants, whereas others are apparently capable of functioning on one or only a few species. If nodule bacteria from one kind of legume produce nodules upon another kind, and vice versa, the organisms of the two plants are said to cross-inoculate. Cross-inoculation tests have defined group relationships among the nodule bacteria of the

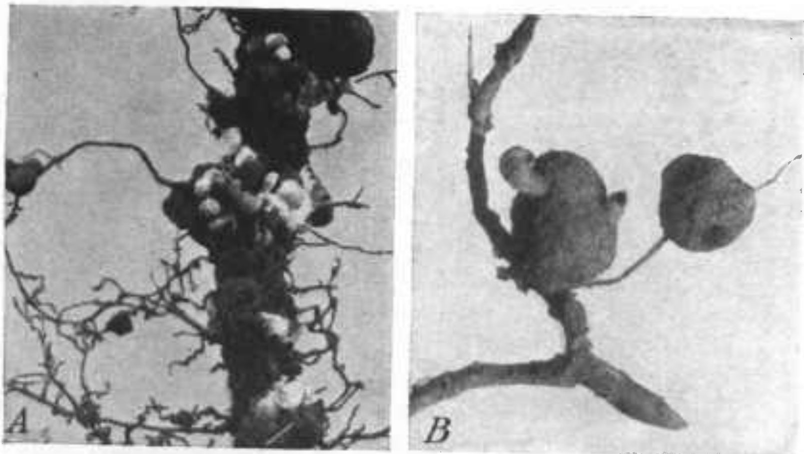

FIGURE 5.—Insect pests on nodules: *A*, Grape mealybugs on soybean nodules; *B*, bean leaf beetle larvae eating cowpea nodules.

common crop legumes. Some of these are shown in the following lists in which legumes which produce nodules with the same race of bacteria are grouped together.

1. ALFALFA GROUP

Common name	Scientific name
Alfalfa	*Medicago sativa*
Bitter clover	*Melilotus indica*
Buttonclover	*Medicago orbicularis*
California bur-clover	*M. denticulata*
Fenugreek	*Trigonella foenum-graecum*
Southern bur-clover	*Medicago maculata*
White sweetclover	*Melilotus alba*
Yellow sweetclover	*M. officinalis*
Yellow trefoil	*Medicago lupulina*

2. RED CLOVER GROUP

Alsike clover	*Trifolium hybridum*
Crimson clover	*T. incarnatum*
Hop clover	*T. agrarium*
Low hop clover	*T. dubium*
Mammoth red clover	*T. pratense perenne*
Rabbitfoot clover	*T. arvense*
Red clover	*T. pratense*
White clover	*T. repens*

3. COWPEA GROUP

Common name	Scientific name
Cowpea	*Vigna sinensis*
Florida beggarweed	*Desmodium purpureum*
Jackbean	*Canavalia ensiformis*
Common lespedeza	*Lespedeza striata*
Korean lespedeza	*L. stipulacea*
Perennial lespedeza	*L. sericea*
Crotalaria	*Crotalaria striata*
Kudzu-bean	*Pueraria thunbergiana*
Lima bean	*Phaseolus lunatus macrocarpus*
Partridge-pea	*Chamaechrista fasciculata*
Peanut	*Arachis hypogaea*
Pigeonpea	*Cajanus indicus*
Hoary tickclover	*Desmodium canescens*
Tepary bean	*Phaseolus acutifolius latifolius*
Velvetbean	*Stizolobium deeringianum*

4. SOYBEAN GROUP

Soybean	*Soja max*

5. GARDEN BEAN GROUP

Garden and navy bean	*Phaseolus vulgaris*

6. VETCH GROUP

Field pea	*Pisum arvense*
Common vetch	*Vicia sativa*
Garden pea	*Pisum sativum*
Hairy vetch	*Vicia villosa*
Broadbean (horsebean)	*V. faba*
Lentil	*Lens esculenta*
Narrowleaf vetch	*Vicia angustifolia*
Purple vetch	*V. atropurpurea*
Sweet pea	*Lathyrus odoratus*

It should be understood that the arrangement of legume bacteria given above is based upon ability to cause nodules to form and that in view of the fact that efficiency in the matter of fixing nitrogen from the air is largely due to a delicate adaptation of the organisms to a particular host, it should not be taken for granted that the organism of any of the species represented will be equally satisfactory for all the legumes in a group. Present knowledge leads to the suggestion that, whenever obtainable, a culture containing strains specific for the particular legume should be used. If they are not available, the relationship shown in the foregoing groups is useful in making selections that may be satisfactory.

NATURAL SOURCES OF LEGUME BACTERIA

The more or less continuous growing of certain leguminous crops helps to maintain the legume bacteria population. Likewise, wild legumes are responsible for the continued presence of certain legume organisms in soils. The cowpea, for instance, is related, from a nodule-bacteria standpoint, to many of the wild legumes in the

South, and therefore it is quite commonly planted without inoculating. On the other hand, since soybean bacteria are peculiarly adapted to soybean varieties only, it is not infrequent that crops of this legume are found without nodules on the roots.

Under conditions in which the legume will make reasonable growth, nodule bacteria do not disappear with the crop. By the decay of nodules, legume bacteria are returned to the soil and even in the absence of the legume may remain there for many years under favorable environment. Considering soils in general, it seems safe to say

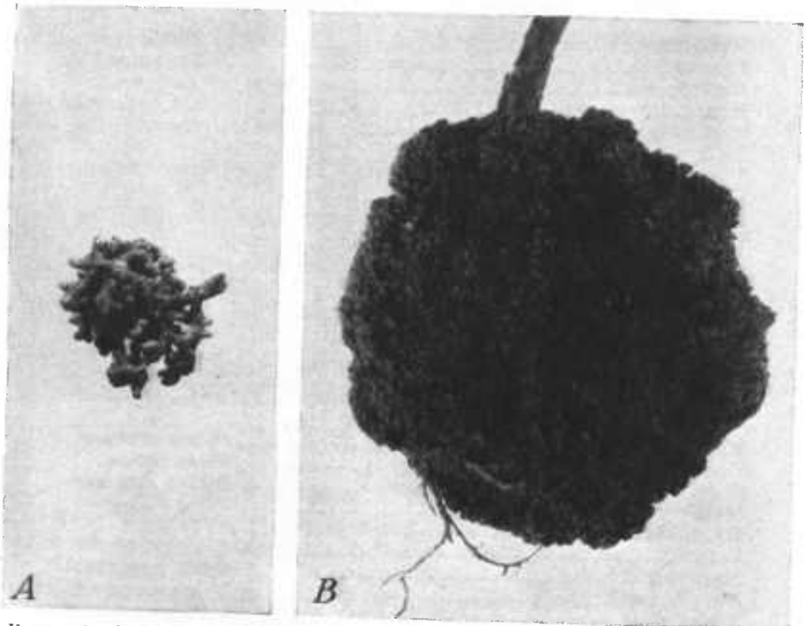

FIGURE 6.—Nonlegume nodules: *A*, From *Cycas* species; *B*, from *Elaeagnus* species.

that nodule bacteria will persist there at least a few years, but whether they will at all times produce the best results can be determined only by trial of a particular crop upon a particular soil.

Legume bacteria may be transferred from one region to another by means of water, wind, animals, farming implements, birds, seeds, insects, etc., but this accidental transference cannot ordinarily be depended upon to give thorough distribution of the organisms. Whether these accidentally distributed bacteria survive and propagate depends upon the environment in which they are placed.

ARTIFICIAL PROPAGATION OF NODULE BACTERIA

By bacteriological methods it is possible to remove bacteria from nodules and isolate them from other bacteria common to plants and soils. Bacteria so isolated are called pure cultures. The establishment of the fact that bacteria are necessary for the growth of legumes, especially on poor soils, that these bacteria have peculiar adaptations to certain legumes only, that they often meet environments in which they cannot live satisfactorily, that they can be obtained in pure

culture and tested for efficiency in nitrogen fixation, and that they can be propagated to almost any degree is responsible for the establishment of laboratories to produce them in large quantities. Bacteria for practical purposes are commonly designated as inoculants or inoculating material, but in seed and other stores they are sold under various trade names.

To produce satisfactory inoculating material for legumes requires specialized training and experience, as well as adequate laboratory equipment for preparing and controlling production. The preparation of cultures of legume bacteria should not be attempted by those without experience or training. The usual process of preparing inoculating material for agricultural purposes consists essentially in selecting and propagating efficient strains of nodule bacteria. Some commercial material is marketed in the form of pure cultures on agar (jelly) in bottles, while much of it is in moist peat or similar substances in various types of cans and boxes. Agar and powder cultures of legume bacteria are illustrated in figure 7.

NONLEGUME INOCULANTS INEFFECTIVE

The unfolding of the relationship between legumes and bacteria prompted scientific attempts to adapt organisms to nonlegumes, but the many efforts in this direction have been without practical sig-

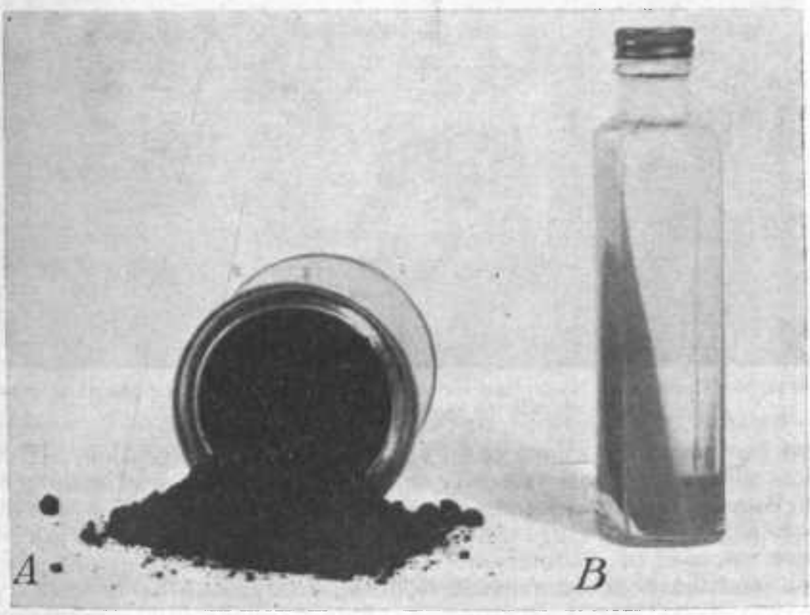

FIGURE 7.—Common types of carriers for legume bacteria; *A*, Moist powder; *B*, agar.

nificance up to the present time. Despite these negative results, there is an occasional promotion of inoculants for the treatment of nonlegumes. These materials are usually made according to a secret or patented formula. The fact that a preparation is patented is not a guarantee of merit. In the last 30 years a number of nonlegume in-

oculants of different origin and composition have been examined, and none have been found to be worthy of practical application.

Certain beneficial soil bacteria are necessary for nonlegumes, but fortunately they are universally present in most agricultural soils. Soils may be replenished with these bacteria and their activities increased by the addition of a liberal supply of organic matter, such as stable or green manure. It is better to depend upon established farming practices to preserve fertility than to spend money for inoculants for nonlegumes from which little or no response can be expected.

IS INOCULATION NECESSARY?

In areas planted to the same legume every year and consistently producing satisfactory crops, inoculation has often failed to show discernible advantage. It appears that in such soils there are frequently abundant organisms of the right kind for inoculation. To test such soil for the presence of these organisms, part of the field

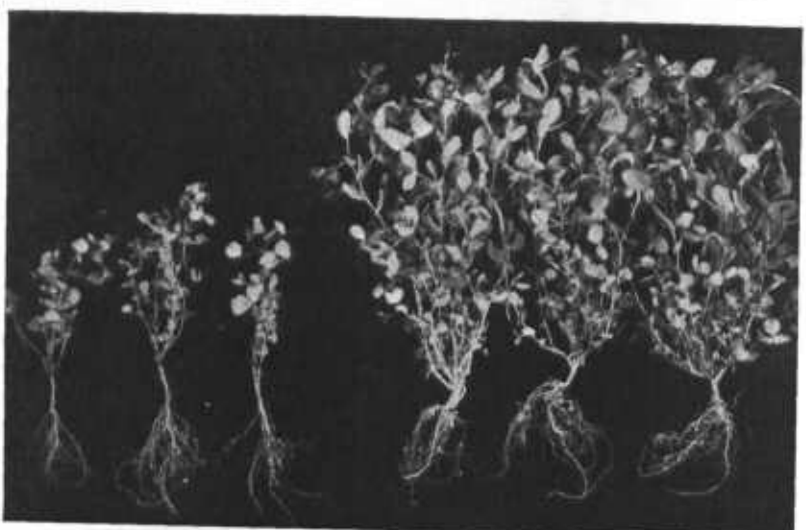

FIGURE 8.—Three plants (left) inoculated by native nodule bacteria, and three (right) inoculated with an inoculant of high efficiency.

may be inoculated and a test area left without inoculation. If the plots selected are satisfactorily uniform, the presence of nodules on the roots in the inoculated area and their absence in the other area, together with better growth of the crop in the inoculated area, will show the need of inoculation. If plants in both areas grow equally well and have adequate root nodules, inoculation is unnecessary. It requires at least one growing season to make this experimental determination.

Even when nodules are produced by organisms already present, a striking improvement in growth may sometimes be obtained by using a selected culture, as is shown in figure 8.

If a particular legume has not been grown on the field for several years, the safest procedure is to apply artificially prepared inoculating material, leaving only a small but representative plot uninocu-

lated, for test purposes. The contrast developed in such a test is shown by figure 9, in which the check plot is paler in color and produced much less forage than the inoculated plots on either side.

The experience of other legume growers in the same neighborhood often furnishes a valuable guide in handling a particular soil type. The county agricultural agent is often especially fitted to give advice based upon wide experience with legumes in the whole area.

CONDITIONS THAT AFFECT LEGUME BACTERIA

Under natural conditions of competition between inhabitants of the soil, legume bacteria have an advantage by their sequestration in nodules from time to time. Without this association, the nodule bacteria must meet adverse soil conditions successfully if they are to survive. Conditions essential to the satisfactory growth of legumes must be fulfilled before maximum results with inoculation can be

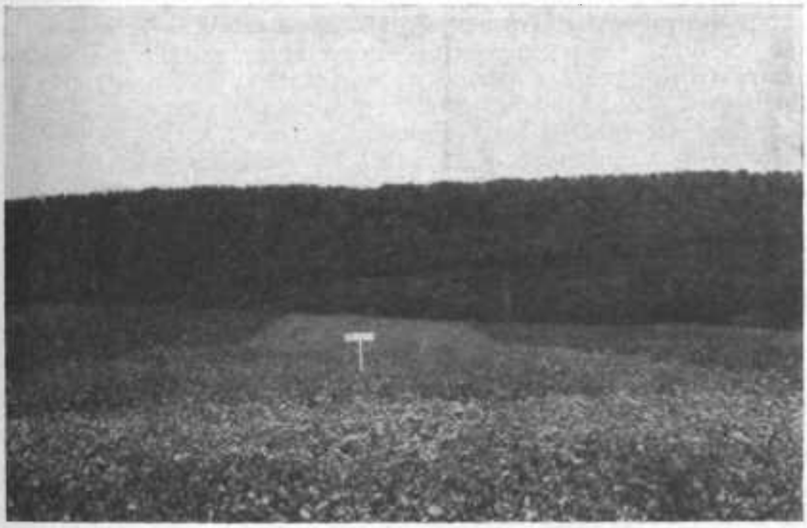

FIGURE 9.—Inoculated and uninoculated soybeans; the light-colored center strip is without nodules.

expected. The principal requirements are the proper preparation of the soil, healthy adapted viable seed, and an available supply of lime, phosphorus, and potash in the soil.

Cultures of nodule bacteria are alive and should be treated as living things. They will tolerate low temperatures much better than high ones. If exposed to a heat that is unbearable by man, or even uncomfortable, the efficiency of the bacteria may be impaired. Inoculating material should be stored in a cool place until used.

Although legume bacteria will tolerate sunlight to some degree, unnecessary exposures either of the unopened containers or of the seed that has been treated should be avoided. Bacteria when dried on seed soon die. If conditions require that inoculated seed be kept a week or 10 days, it is advisable to reinoculate. For this reason the purchase of preinoculated seed is inadvisable.

Seed treated with legume bacteria should not come into direct contact with caustic lime, seed disinfectants, or concentrated fertilizers. Ordinarily, not much harm is to be expected from sowing inoculated air-dried seed and fertilizer from their respective drill compartments through the same tubes. Appreciable injury may occur if the seed is sown in a damp state with fertilizer. The obvious procedure in such cases is to drill fertilizer and seed at different times.

Once nodule bacteria are in the soil, they are subject to the conditions existing there; if the reaction of the soil is suitable and sufficient moisture and plant food are present, they should function normally. Acid soils, in accordance with the degree of acidity and the ability of the nodule bacteria to tolerate this condition, tend to eliminate these organisms. Nodule bacteria of alfalfa, sweetclover, red clover, and vetch are representative of those that are not very acid-tolerant, while soybean, velvetbean, cowpea, lespedeza, and lupine bacteria belong to the acid-tolerant types.

It is desirable that fresh inoculating materials be obtained whenever possible. On packages of commercial material it is usually customary to designate a period of usefulness for the bacteria after which they should not be used. In purchasing inoculants, be sure that they are labeled by the manufacturer for the legume to be planted and that they are well within their efficient period.

METHODS OF APPLYING BACTERIA TO SEED AND SOIL

A procedure for treating seed with inoculation, to be satisfactory must place the bacteria where the roots begin to form. Usually each package of inoculant contains directions for use. These should be followed explicitly, because they represent the experience of the manufacturer with his product. If, as a result of treatment, the seed swells slightly, causing the rate of planting to decrease, the seeding apparatus may be adjusted to deliver seed faster to compensate for this retardation.

Although at one time advocated as the principal means of securing inoculations for new areas, the transfer of field soil has been largely superseded by packaged cultures, which are easy to transport and apply and which, if properly made and handled, usually give satisfactory results. If transfer of field soil seems desirable, it should be collected from a field known to contain the proper organisms and to be free from crop diseases. The soil is sieved and drilled on the field like fertilizer. In small quantities it may be mixed with the seed with or without liquid and sown with them.

Under some conditions it is desirable to treat a crop already planted. This may be accomplished by drilling, preferably just before a rain, naturally inoculated soil or soil mixed with artificially prepared inoculants. If it can be done without injury to the crop, the inoculating material should be incorporated in the soil, since legume bacteria travel very slowly and in close-textured soils may stay where they are dropped.

Legume bacteria cultures are ordinarily maintained in moist carriers and are applied to seed with the addition of water. Drying is detrimental to these organisms. Hence after inoculation prompt planting is necessary. For a few years inoculants for use without

water have been sold; they have been found less consistently satisfactory than comparable materials applied with water.

TESTING LEGUME INOCULANTS

For the protection and guidance of farmers and others, a few States and the United States Department of Agriculture inspect commercial inoculating materials every year. Samples of each brand of materials for the common leguminous crops are collected from various sources in the United States and tested for ability to produce nodules and to stimulate plant growth.

With some legumes, principally soybeans, peas, and alfalfa, it is sometimes possible to find field soils free from their nodule organisms; and, as shown in figure 10, striking results are sometimes obtained. However, the lack of assurance that the results will be

FIGURE 10.—*b*, Inefficient cultures produce yellowish plants (center), while *a* and *c*, good inoculants, give plants a dark green color (right and left).

conclusive in the field makes it advisable to use greenhouse tests, since under these conditions a better control may be had over the various factors. Briefly, the process of testing consists of inoculating seed according to directions and planting in sterile sand. Great care is necessary to prevent the entrance of nodule bacteria from without and the transfer of bacteria from one pot to another. When a culture under these conditions does not produce nodules it is considered unsatisfactory.

Pure cultures may be tested for efficiency by greenhouse tests, and selections of superior organisms may be made from the results obtained. As among other living things, there are found in the nodule bacteria family drones or parasites, moderately efficient cultures, and those that are exceptionally efficient. Nodule bacteria cultures of varying degrees of efficiency may produce nodules, but the effect on the plant may be great, little, or detrimental, in accord-

ance with the quality of the germs that caused them. Although apparently of rather rare occurrence, harmful legume bacteria have been reported. The effects of harmful bacteria will be noted in the crop. Hence, if in greenhouse tests a culture causes nodules to form but fails to cause proper plant growth in comparison with other cultures, it is regarded as unsatisfactory.

FERTILIZING VALUE OF LEGUMES

Much nitrogen is added to the soil by the use of legumes as green-manure crops and, in a lesser degree, by careful preservation and return to the soil of manures from feeding harvested legumes. Just what quantity of nitrogen may be brought to the soil by either means depends upon many factors, important among which are climate, the physical and chemical condition of the soil, its richness in elements necessary for growth, the quality and variety of legume seed planted and its adaptability to the soil and climate, and the efficiency of the nodule organisms.

However, if a crop of legumes is removed, much nitrogen goes with it, and the benefit to the soil derived from the legume depends upon the amount of nitrogen in the stubble and roots. This may vary considerably according to conditions of growth, but it is roughly estimated that the roots and stubble may represent from one-eighth to one-third of the total plant material. Since the top growth is usually richer in nitrogen than the roots, even if one-third of the plant is represented by roots and stubble, less than one-third of the nitrogen is left in the soil. In addition to supplying some organic nitrogen, roots and stubble also improve the physical condition of the soil and in decaying stimulate the activities of other bacteria which are essential to keep the soil in condition for plant growth.

The value of legumes for forage, seed, food, industrial purposes, and soil enrichment has caused them to be planted widely. About 50 million acres in the United States are devoted each year to their growth, and it is estimated that they contribute annually between 1 and 2 million tons of nitrogen from the air. Since much of the soil in the United States is losing fertility through overcropping to crops other than legumes and by erosion, it is evident that, in the interest of soil-fertility maintenance, greater areas should be planted to leguminous crops in the future, and nodule bacteria will play an important role in insuring the highest efficiency from these crops.

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